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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
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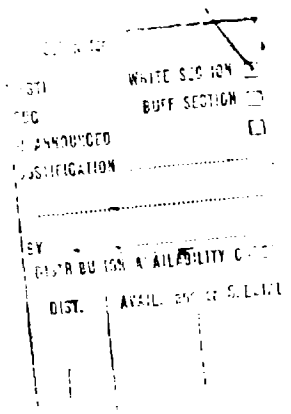

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FOREWORD
(Nontechnical summary)

Many of the ill effects observed in mammals subjected to radiation doses where survival is probable are caused by injury to the blood cell forming system. The cells of this system have a finite life-span (e.g., 120 days for human red cells) and are renewed continuously. In order to maintain their normal number, they must reproduce by cellular division. This process is highly sensitive to ionizing radiation since it is an established fact that cells which reproduce continuously at a high frequency are more severely damaged even at low radiation dose levels.

Figure F-1 depicts the blood cell forming feedback system. Since we have a better understanding of the red cell system, it will be briefly described here, however, it may be assumed that a similar mechanism controls white cell formation. Adult red cells have the sole function of carrying oxygen to cells for metabolism. It has been postulated that, when a small number of these red cells expire, a minutely decreased amount of oxygen would be supplied to specific cells located in the kidney. In response to this intracellular change in partial oxygen pressure, these cells produce and release a hormone into the blood called erythropoietin. This hormone

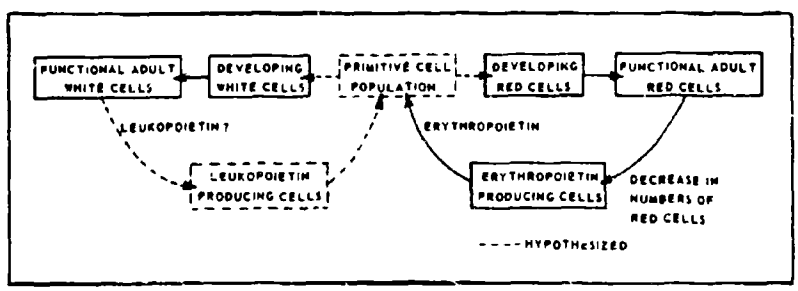

Figure F-1. Blood cell forming feedback system.

stimulates cells from the primitive population, located in the bone marrow, to transform them into developing cells, which, after about three divisions and further development, eventually become functional adult cells. Under normal conditions in healthy animals, this physiological feedback system maintains a steady number of red blood cells. Ionizing radiation destroys a number of these cells in the primitive and developing cell compartments, and may lead to illness and even death.

In the present study, an attempt was made to assess the damaging effects of three radiation doses from two different sources (x-ray machine and mixed gamma-neutron radiation from the AFRRI-TRIGA reactor) upon the primitive cell population. Rats were infused with red cells obtained from other rats until their circulation was overloaded with them and the cells in the kidney stopped producing erythropoietin. When this occurs, the primitive cell population becomes dormant and no new red cells are produced. However, upon the injection of a prescribed quantity of hormone, the primitive cells will respond, this response being used as a measure for normal red cell production. When ionizing radiation damages or destroys these primitive cells, obviously a decreased number is available to respond to the hormonal stimulation. The incorporation of radioiron into newly formed red cells was utilized as a measure of cellular production.

It was observed that the amount or degree of injury to the primitive cell population (also called stem cells) increased with increasing radiation dose. Also, the rate of recovery, with increasing time after irradiation, was fluctuating.

Injury and recovery appeared to be of the same degree with a given dose regardless of whether the animals were irradiated with x- or mixed gamma-neutron radiation at the same dose.

It was further observed that animals which had been irradiated previously did not recover as rapidly from a second radiation exposure.

If one could assume that ionizing radiation injures man as it does rodents, then it could be postulated that a second exposure occurring from the 3rd to the 6th day after the first irradiation may be less damaging than from the 6th to the 9th day, due to inherent physiological adjustments. However, before such a definite conclusion can be made, some additional studies with other mammalian species should be conducted to demonstrate interspecies similarities in response to the injurious effects of ionizing radiation.

ABSTRACT

It is now well established that many ill effects observed in mammals exposed to radiation doses where survival is probable are caused by damage to the hematopoietic system. In recent years scientific discoveries have slowly advanced the knowledge of the physiological basis underlying the response of the erythropoietic system to ionizing radiation. Nevertheless, a number of problems remain to be studied and certain of these are the subject of this report.

The present experiment was designed to obtain recovery and residual injury data on hematopoietic stem cells of 6 groups of polycythemic rats exposed to one of three relatively low doses (150, 200, and 250 rads) from one of two radiation sources.

The radiation sources were a 250 kVp x-ray generator and the AFRRI-TRIGA Mark F reactor. About 60 percent of the dose produced by the reactor was from gamma rays; the remainder was from neutrons.

In the polycythemic rats, the response of hematological stem cells to erythropoietin as measured by ^{59}Fe uptake in the progeny of these cells, was utilized as a measure of the effects of the irradiation. Peripheral leukocyte counts were obtained to assess competitive stimulation of related blood cells upon the hypothesized multipotential stem cell pool.

It was observed that stem cell injury increases significantly with increasing radiation dose as expressed in the first rapid recovery phase. Although recovery responses are dose dependent, they are independent of the type of radiation and, in general, the RBE of gamma-neutron radiation appears to be 1. A significant decrease in the recovery response is observed in animals subjected to a repeated dose

of 200 or 250 rads after a 90-day rest period. Leukopoietic recovery, as noted in the circulatory blood, begins approximately 8 days after exposure.

The data suggest that the postirradiation physiological adjustments in the rat initiated by recovery and subsequent cellular proliferation result in the early abortive cellular rise and its termination.

In this investigation, the residual injury observed after a repeated radiation exposure could be the result of faulty recovery in a number of primitive progenitor cells. However, the possibility of a reduction in numbers due to anatomical changes in the internal environment must be considered.

I. INTRODUCTION

The ill effects primarily observed in mammals exposed to radiation doses where survival is probable, are those caused by damage to the hematopoietic system.[7] Survival depends greatly on the inherent capacity of this system for repair. Extensive studies have been conducted in the past to elucidate the mechanisms involved in radiation damage and subsequent recovery of the hematopoietic system.[6,15,22,24,25] In recent years scientific discoveries have slowly advanced the knowledge of the physiological basis underlying the response of the erythropoietic system to ionizing radiation. In the rat, exposure to a sublethal radiation dose causes an immediate decrease in the number of erythropoietic precursor cells which is followed by a rapid increase in 24 to 48 hours.[17,23] This initial or "abortive" rise is terminated and a secondary fall in precursor cells is noted between the 5th and 6th postirradiation day. Several oscillations may follow before an apparent return to normalcy occurs. The initial recovery rise in erythrocytic cells and the subsequent oscillations are caused by the incapability of the injured stem cell population to release a sufficient number of cells for erythropoietic recovery while attempting to replace cells killed by radiation through homomorphogenic division.[3] Further modifications during the precursor phase must also be considered.[1,27]

Finally, several studies in recent years have presented evidence that while postirradiation erythrocytic repair is sufficient to permit, under normal conditions, a return to the preirradiation cellular turnover rate, a small fraction of injury sustained in the stem cell system is irreparable.[2,4,5]

Although these newly acquired findings on the postirradiation erythropoietic recovery kinetics in the rat advanced our knowledge, a number of problems remained to be studied. Some of them are related to the possible dose dependency of initial injury and subsequent recovery as well as nonreparable injury, while others need clarification in terms of a potential RBE difference from different qualities of radiation sources. The present study was designed to obtain recovery and residual injury data of hematopoietic stem cells in polycythemic rats exposed to three relatively low doses of either 250 kVp x ray or mixed gamma-neutron radiation. The capability of stem cells in irradiated polycythemic animals to respond to known quantities of erythropoietin was utilized as a measure of their number. The validity of this assumption has been discussed in previous publications.[3,16,21] Furthermore, a comparison was made with leukocytic production in order to establish a possible relationship of the progeny of multipotential stem cells.

II. METHODS

Male Sprague-Dawley rats obtained from the colony of the Simonsen Laboratories, White Bear, Minnesota were used in this study. The rats were 140 ± 3 days old when the experiment was initiated and had a mean weight of 329 ± 45 g. The animals were housed individually in clean wire cages, with a wire mesh bottom. They were maintained with a minimum of handling and allowed free access to biscuits obtained from the D and G Laboratory Animal Foods Company, and water.

The animals were divided into two groups, each comprised of 283 rats. One group was exposed to x- and the other to mixed gamma-neutron radiation. One-third

of each group was subjected to 150 rads, while the second third was exposed to 200 rads and the remainder to 250 rads. A nonirradiated control group of 96 rats was subjected to the same treatment regime as those irradiated except for the radiation exposure.

All rats were made polycythemic by the intravenous injection of four doses of 5 ml washed homologous erythrocytes. The cells were injected on the 10th, 7th, 5th, and 3rd day prior to irradiation. In order to maintain polycythemia in rats from the 8th to the 11th postirradiation day, a fifth dose of 5 ml washed homologous erythrocytes was administered on the 6th day after radiation exposure. On the day of radiation exposure and on each of the following 11 days, a new set of 8 rats from each dose group was injected via tail vein with 10 units of Erythropoietin A, Step 1, obtained from the Connaught Medical Research Laboratories, Toronto, Canada. At the time of the erythropoietin injection, the hematocrits of the animals ranged from 58 to 68 percent. Two days after erythropoietin administration, at a time when previous experiments[3] had indicated that precursors newly differentiated from stem cells were optimally synthesizing hemoglobin, the rats were injected intravenously with a sodium citrate-buffered $FeCl_3$ solution containing 1 μCi of ^{59}Fe in 0.01 μg of total iron. One week after the radioiron injection, blood was withdrawn from each rat via the jugular vein. Of this blood, 0.05 ml was pipetted into a stoppered test tube to which 2 ml of distilled water were added. The radioactivity of these samples was counted in an automatic dual-channel, well-type, gamma scintillation detector using a 3 x 3 thallium activated sodium iodide crystal. To determine the total ^{59}Fe activity in the blood, measurements for blood volumes

were conducted utilizing ^{51}Cr labeled erythrocytes as described by Sterling and Gray.[26]

To measure the radiation effects upon leukopoiesis in the polycythemic rats, peripheral leukocyte counts were obtained daily for 18 days after exposure.

Upon completion of all the experimental procedures, the animals were permitted to rest for a 90-day period. At the end of this time, the rats which had been previously exposed to the quantity and quality of radiation described above were irradiated again with identical doses from the same sources. This was a test for residual injury. All other experimental procedures utilized after the first exposure were repeated in the irradiated rats, as well as in the nonexposed controls.

The x-ray source was an x-ray generator with the following physical parameters: 250 kVp, 30 mA, 0.95 mm Cu + 1.2 mm Be filtration (HVL-1.9 mm Cu); and target to subject midline distance 110.5 cm. The midline exposure rate was 20 R/min in air. For the radiation exposure, all rats of an exposure group were placed in Lucite boxes and arranged in the radiation field so that the tissue dose rate to the midline of the exposure volume was similar for all rats (maximum deviation ± 4 percent).

The swimming pool type AFRRI-TRIGA reactor was the source for the mixed gamma-neutron radiation. (The description of the exposure room, dosimetry, and radiation field may be found in a previous report.[11]) The rats were placed in the exposure room so that they were in a radiation field with an isodose surface 292 cm from the reactor center line. The midline tissue dose rate, as measured in a small unperturbed tissue sample which was in charged particle equilibrium and surrounded

by air, was 20 rads per minute; the ratio of gamma dose to neutron radiation dose contribution was 1.5.

A tissue equivalent plastic chamber with a flow of tissue equivalent gas and a graphite chamber with a carbon dioxide flow were utilized for primary dosimetry. The concepts and techniques involved in this procedure are described in the International Commission on Radiological Units and Measurements (ICRU) Report 10b (National Bureau of Standards Handbook 85).[20] The dosimetry for each radiation exposure was based on the integrated current from the tissue equivalent chamber. In addition, silver phosphate glass microdosimeters to monitor the gamma dose and sulfur tablets for a measurement of the neutron dose were utilized.

Depth dose measurements were made in a Lucite phantom using miniature tissue equivalent ionization chambers as described by Chambers.[9]

To test the significance of effects with increasing radiation doses and with repeated exposures, the statistical ranking test as devised by White[29] was utilized.

III. RESULTS

With increasing radiation dose from either exposure source, rats show a significant decrease in ^{59}Fe incorporation during the initial rapid recovery phase (Figures 1, 2, 3, and 4). Animals irradiated with 150 rads of x rays once or twice and those subjected to 150 rads mixed gamma-neutron radiation show maximum cellular recovery 4 days postirradiation and secondary decreases thereafter. In all cases for this radiation exposure, ^{59}Fe values of irradiated rats exceed those of the controls at the time of the first recovery peak. Animals exposed twice to 150 rads

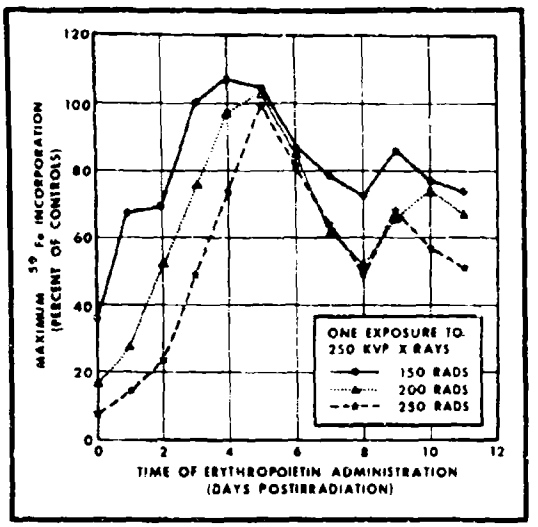

Figure 1. Stem cell recovery in rats exposed to three sublethal doses of x radiation

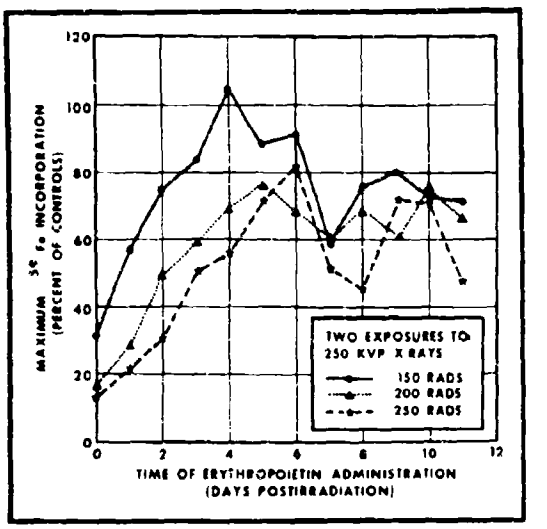

Figure 2. Stem cell recovery in rats exposed to a repeated sublethal dose of x radiation

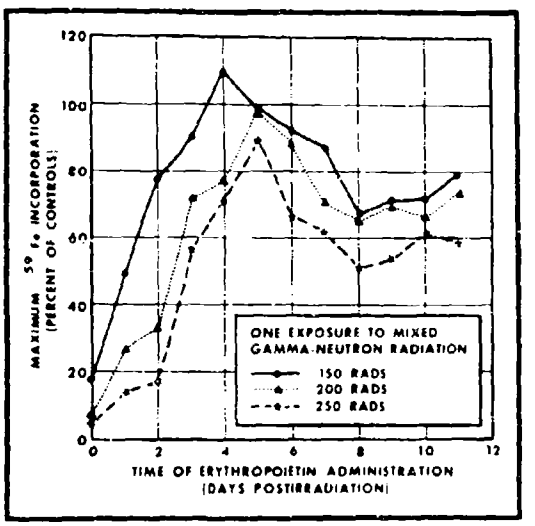

Figure 3. Stem cell recovery in rats exposed to three sublethal doses of mixed gamma-neutron radiation

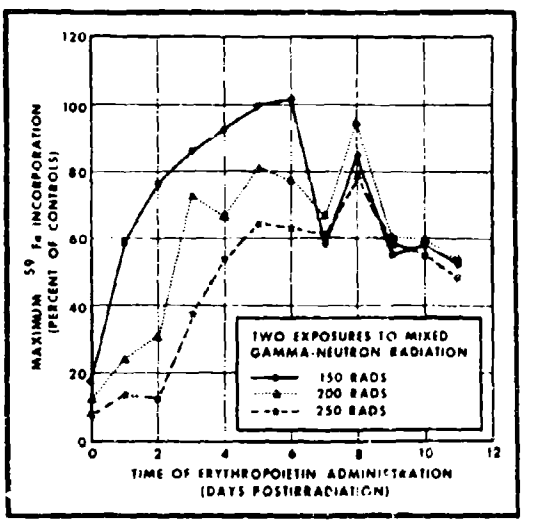

Figure 4. Stem cell recovery in rats exposed to a repeated sublethal dose of mixed gamma-neutron radiation

gamma-neutron radiation reach their peak value 2 days later, at which time their ^{59}Fe incorporation approximately equals that of the controls.

Maximum recovery is not obtained until the 5th postirradiation day for the higher radiation doses, and for rats exposed for the 2nd time to 250 rads x ray, this did not occur until th· 6th day. Overshoots were not observed for rats exposed to 250 rads from either radiation source.

During the secondary decreases and subsequent oscillations, the dose dependency becomes less distinct. However, it can still be noted, particularly after the first exposure from either type of radiation source.

Although the recovery responses are dose dependent, Figures 5, 6, and 7 indicate that, in the main, they are independent of the radiation source and the RBE

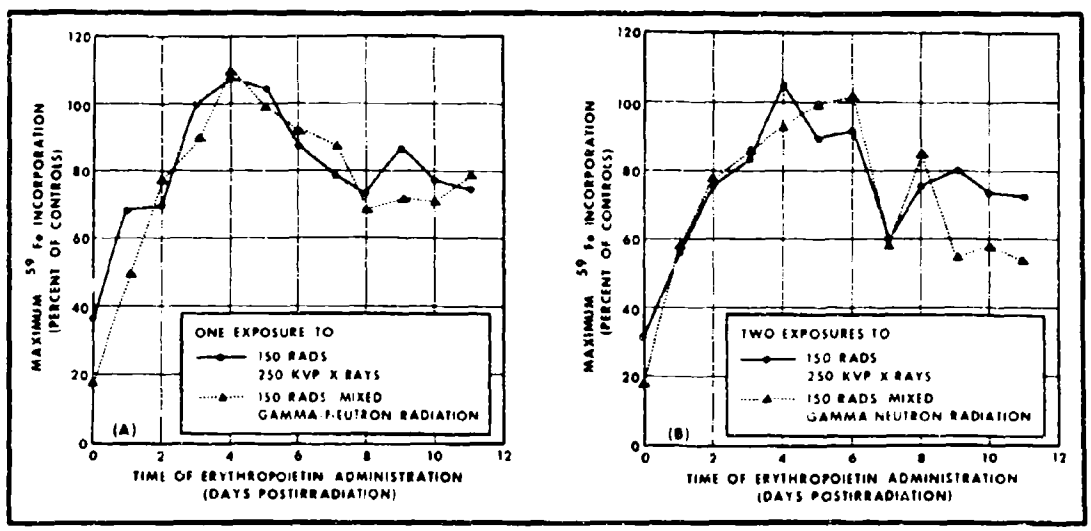

Figure 5. Comparative stem cell recovery in rats exposed once or twice to 150 rads of x ray or mixed gamma-neutron radiation

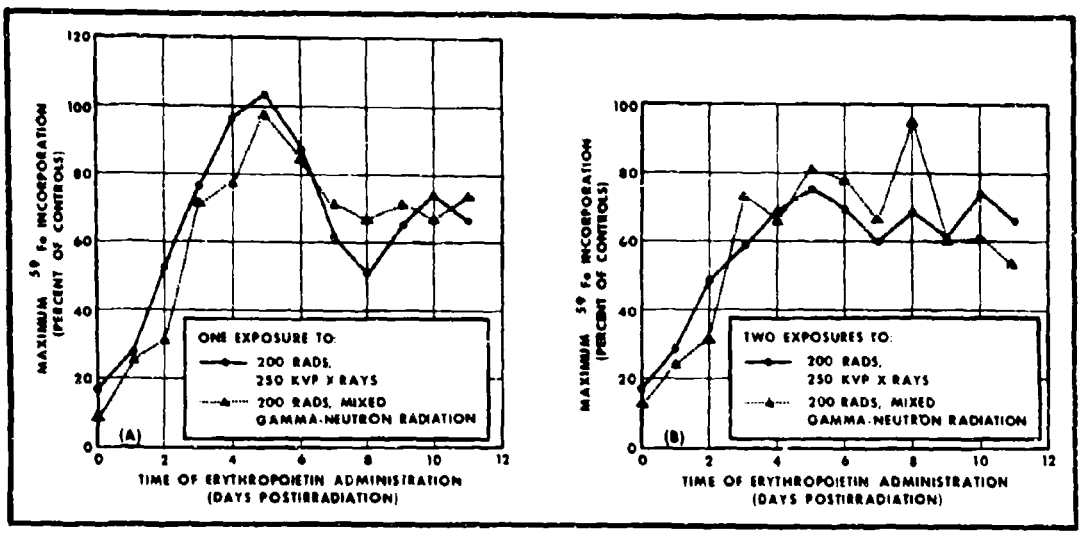

Figure 6. Comparative stem cell recovery in rats exposed once or twice to 200 rads of x ray or mixed gamma-neutron radiation

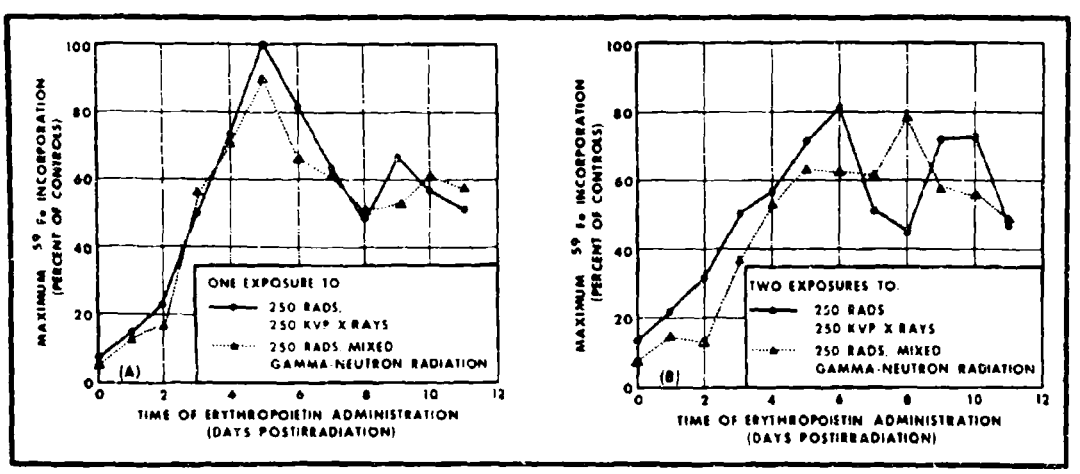

Figure 7. Comparative stem cell recovery in rats exposed once or twice to 250 rads of x ray or mixed gamma-neutron radiation

of mixed gamma-neutron radiation for this end point appears to be 1. For one radiation exposure to 200 rads, x ray appears to have been less injurious as measured by the recovery response. However, only the comparative mean values obtained on the 4th postirradiation day are significantly different ($p \leq 0.05$).

Figures 8, 9, and 10 show that the effects of two radiation exposures appear to be greater than that of the initial exposure for all doses from either radiation source as measured during the first rapid recovery phase. Significant differences ($p \leq 0.05$) are noted for 200 (days 3-6) and 250 rads x ray (days 4-5) and for 250 rads mixed gamma-neutron radiation (days 3-5).

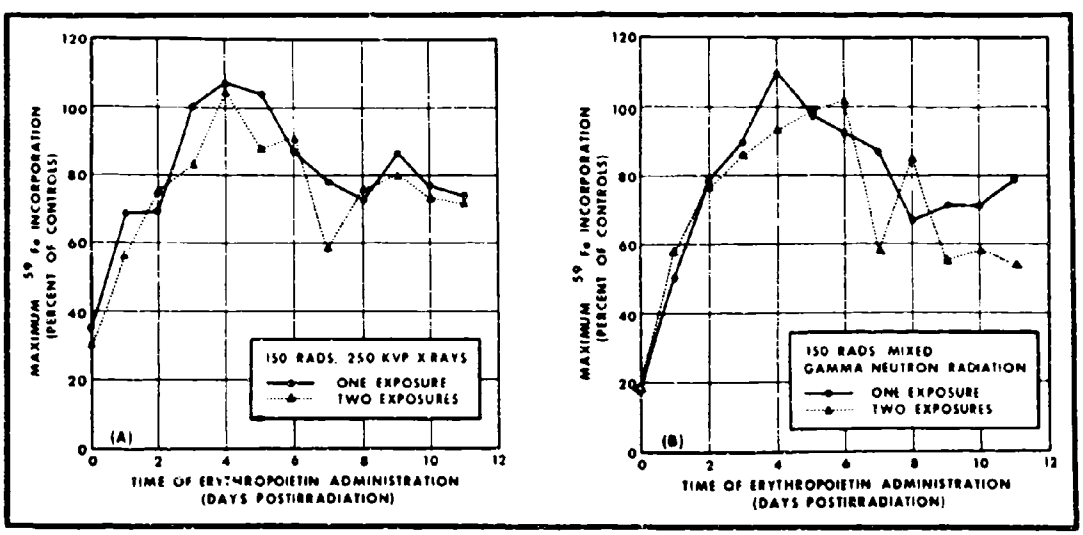

Figure 8. Residual injury in rats exposed twice to 150 rads x ray or mixed gamma-neutron radiation

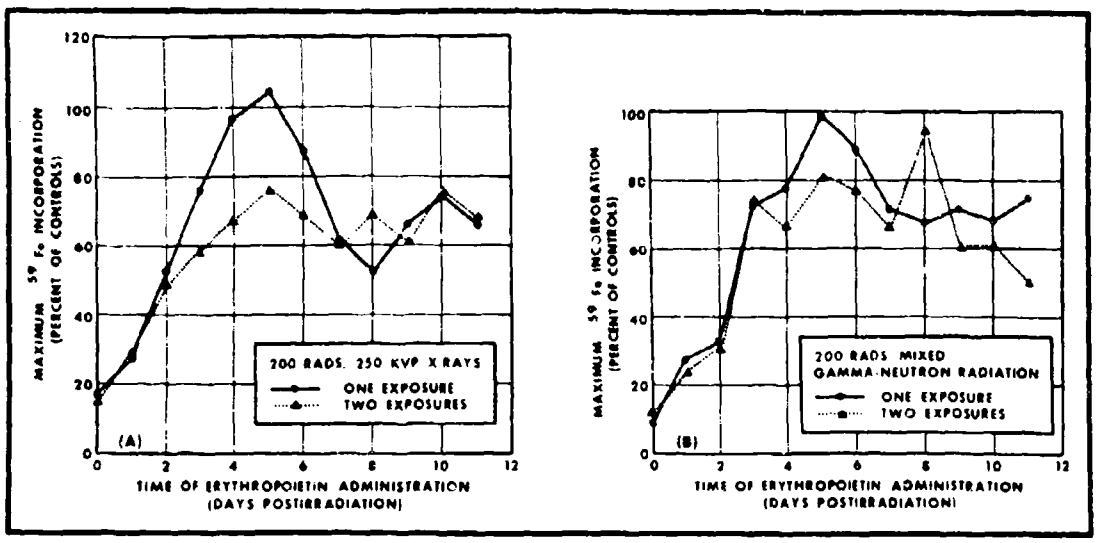

Figure 9. Residual injury in rats exposed twice to 200 rads x ray or mixed gamma-neutron radiation

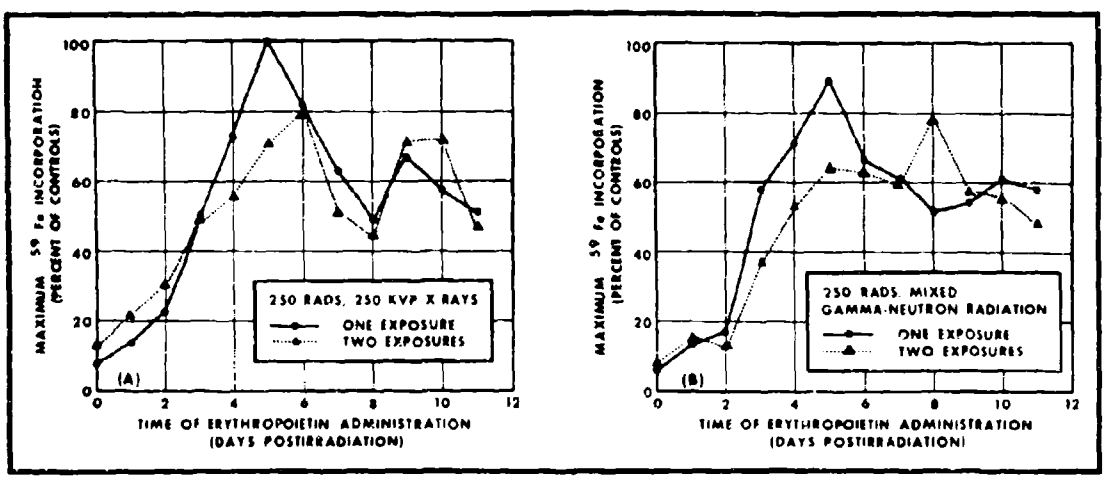

Figure 10. Residual injury in rats exposed twice to 250 rads x ray or mixed gamma-neutron radiation

Figure 11 demonstrates the effect of the three different radiation doses upon leukopoiesis as assessed by peripheral cell counts. The cellular decrease in animals exposed to 150 rads of either source appears to have been smaller as compared with that at higher dose levels. The RBE of gamma-neutron radiation appears to be 1. In all cases, the peripheral white cell count approaches maximal depression within 24 hours and appears to remain at that low level until the 8th day. Recovery appears to commence approximately on the 8th day for all dose groups from either radiation source. Assuming a bone marrow white cell precursor transit time of 2 days,[7] it suggests that leukopoietic recovery was initiated when erythropoiesis commenced its secondary depression.

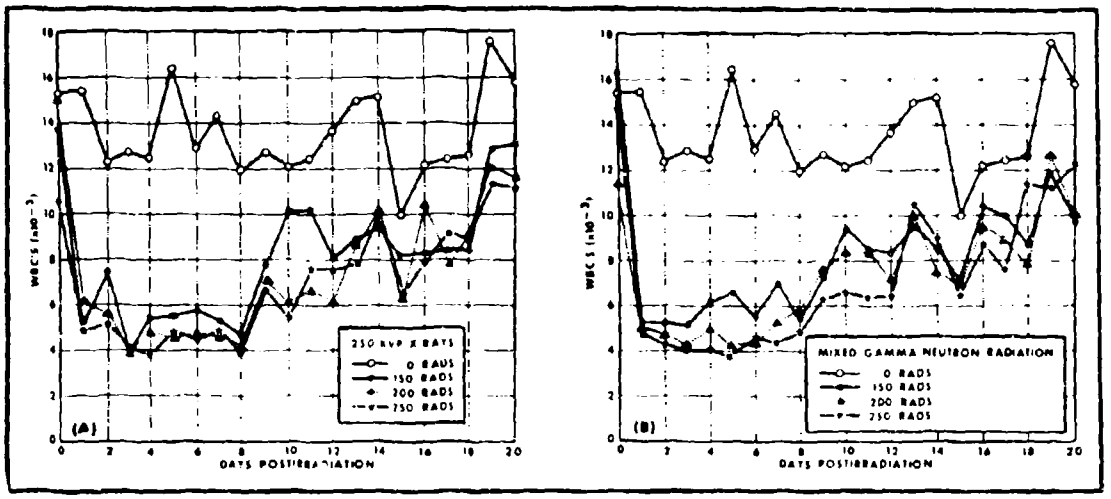

Figure 11. Peripheral leukocyte counts in rats exposed to one of three sublethal doses of x ray or mixed gamma-neutron radiation

IV. DISCUSSION

The results of the present experiment support the hypothesis of postirradiation recovery and residual injury in the hematopoietic stem cells developed in earlier reports.[3,4] The new findings, added to what has been established previously, permit the presentation of a schematic model as depicted in Figure 12. Although stem cells are often considered to be a uniform group of cells, more recent evidence exists which suggests that there are at least two functionally different populations.[8] The first is comprised of the uncommitted multipotential primitive progenitor cells which transfer into a potentially committed group of cells after appropriate intracellular changes. The latter group of cells will be available for differentiation into erythrocytic precursors when stimulated by erythropoietin,[1,14] or into the other hematological precursors upon other appropriate physiological stimuli. As depicted in Figure 12, it is further postulated that only a small fraction of the potentially committed group is activated for normal erythrocyte turnover. This active fraction may expand under emergency conditions such as hemorrhage, but only as long as there exists a certain minimum number of inactive cells to support it. Of course, one of the functions of the inactive cells is to maintain and control their cellular space. Should the inactive stem cell population be decreased, the capacity for cellular turnover will diminish correspondingly.

Immediately after irradiation there exist intact, injured and killed stem cells.[3,7] Since the data of the present study show that injury as well as recovery was dose dependent, it may be assumed that the proportion of each type is related to the radiation dose employed and presumably the types are uniformly distributed

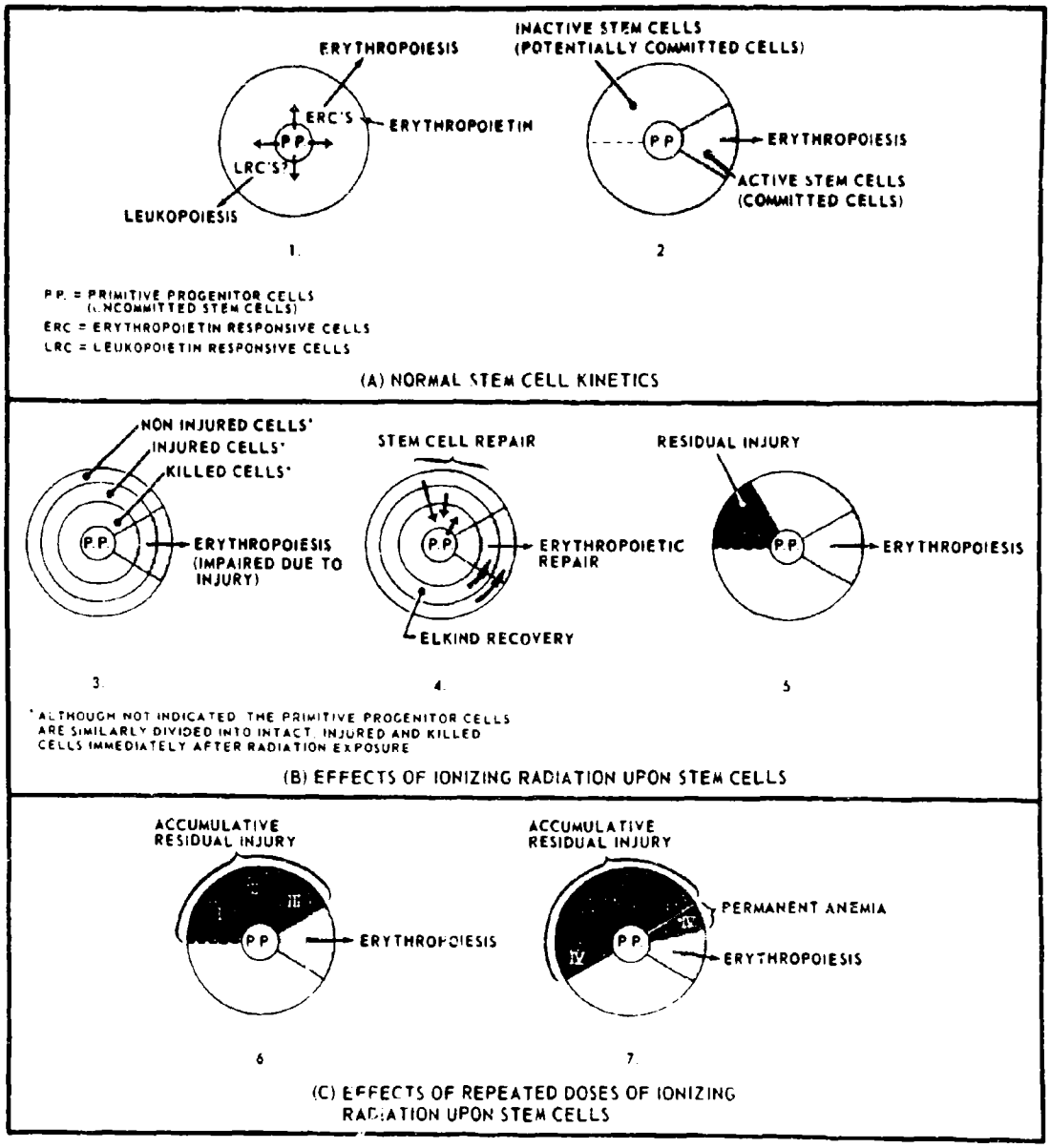

Figure 12. Schematic model of normal stem cell kinetics and changes induced by ionizing radiation

throughout the primitive progenitor pool. There is a shift toward greater numbers of injured and killed cells with increasing radiation dose and it appears that gamma-neutron radiation might have been more effective. The net effect is an impairment in erythropoiesis. Recovery is initiated immediately and is complete in a few hours.[12,13] It is suggested, however, that not all cells return to preirradiation normalcy.

It has been proposed that the noninjured stem cells undergo homomorphogenic division to replace the killed cells, while injured cells, incapable of self-renewal but not of heteromorphogenic division, are responsible for the first rapid cellular recovery also called "abortive rise".[7,18] The secondary cellular drop, as seen in the present experiment and which had also been observed in other studies,[3,17,23] is then ascribed to the complete utilization of these injured cells. While such as interpretation might be attractive, the results of the present experiment do not support it. Although more injured cells are produced by exposure to higher doses, the rate of the "abortive rise" is not correspondingly greater. As a matter of fact, it is either similar or slightly less pronounced with increasing radiation doses. Peripheral leukocytosis commences approximately on the 8th postirradiation day in the present experiment. Assuming a 2-day precursor transit time, recovery in the bone marrow must have started when the initial rapid rise for erythropoiesis was terminated, about the 5th or 6th postirradiation day. Studies of postirradiation myelocytic changes in rat bone marrow seem to substantiate this assumption.[19] This would then suggest that competitive stimulation for leukocytic recovery decreased the availability of

multipotential stem cells for erythropoiesis and at least contributed to the termination of the "abortive rise".

The data of the present study suggest that there exists a number of complex physiological adjustments, in the radiation injured rat, which results in early recovery, "abortive rise", and its termination. No single response can account for all events. It is proposed that recovery in the primitive uncommitted stem cell group, as well as in the potentially committed one, is accomplished by homomorphogenic division of intact, as well as by partly or completely repaired radiation injured cells (Figure 12). Similarly, erythropoietic repair as seen in the "abortive rise" is accomplished by differentiation of intact and injured cells. The termination of this initial, rapid recovery response could be caused by a combination of at least three events and conceivably, there could be several more. First, a recent study[8] revealed that the replacement of potentially committed cells may occur at a slower pace than their removal for erythropoiesis. Next, some of the injured cells may, indeed, only have the capability to differentiate and are phased out.[7,18] Finally, as suggested by the data from the present study, competitive stimulation for leukocytic repair may decrease the number of stem cells available for erythrocytosis.

The termination of the initial rise is followed by a diminished cellular response to erythropoietin for the next 2 to 3 days. This may indicate that an insufficient number of stem cells were available during this time to respond to the demand of the total hematological system. The secondary oscillations may result from periodic changes in competitive stimulation for different types of blood cells prior to the system's return to apparent preirradiation normalcy.

As depicted in Figure 12, it is postulated that stem cell recovery is incomplete after an exposure to ionizing radiation. However, since this residual injury is relatively small, it presumably, can be measured only during the initial period after a second radiation exposure. This was verified in a previous experiment[4] after repeated exposures to 300 rads x ray and in the present study in rats subjected to a repeated exposure of 200 or 250 rads from either source of radiation. The residual injury could be the result of faulty intracellular recovery in a number of primitive progenitor cells. The progeny of such cells might be responsive to erythropoietin, but never differentiate into mature erythrocytes. However, it is conceivable that the stem cell population was reduced physically in numbers due to anatomical changes in the internal environment. Such a possibility was discussed in a recent article.[10]

When radiation doses are repeated a sufficient number of times with intervals for recovery between exposures, the accumulated residual injury results in a significant reduction of the number of inactive, potentially committed cells (Figure 12) This should then result in a decrease of erythropoiesis. Indeed, permanent anemia was observed under such conditions in an earlier experiment.[2]

It is of interest to note that the RBE of gamma-neutron radiation is 1 or at least very close to 1. This is in contrast with the results of comparative survival studies conducted with similar strains of rats exposed either to mixed gamma-neutron radiation or 250 kVp x ray.[28] It was observed that the $LD_{50/30}$ was greatly reduced in rats exposed to mixed gamma-neutron radiation (RBE 1.7). Although injury to the hematopoietic system is the primary lesion noted at sublethal exposures, it is quite clear that it alone does not account for the reported differences in survival. The

known detrimental effect of neutrons upon the gastrointestinal system, permitting secondary infections, probably provides the additive factor.

V. CONCLUSION

In polycythemic rats, the response of hematological stem cells to erythropoietin, as measured by ^{59}Fe uptake in the progeny of these cells, was utilized as a measure of the effects of irradiation from a 250 kVp x-ray generator and the AFRRI-TRIGA Mark F reactor. Peripheral leukocyte counts were obtained to assess competitive stimulation of related blood cells upon the hypothesized multipotential stem cell pool.

It was observed that stem cell injury increases significantly with increasing radiation dose as expressed in the first rapid recovery phase. Although recovery responses are dose dependent, they are independent of the type of radiation and, in general, the RBE of gamma-neutron radiation appears to be 1. A significant decrease in the recovery response is observed in animals subjected to a repeated dose of 200 or 250 rads after a 90-day rest period. Leukopoietic recovery, as noted in the circulatory blood, begins approximately 8 days after radiation exposure.

The data suggest that the postirradiation physiological adjustments in the rat initiated by recovery and subsequent cellular proliferation result in the early abortive cellular rise and its termination.

In this investigation, the residual injury observed after a repeated radiation exposure could be the result of faulty recovery in a number of primitive progenitor cells. However, the possibility of a reduction in numbers due to anatomical changes in the internal environment must be considered.

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13 ABSTRACT

It is now well established that many ill effects observed in mammals exposed to radiation doses where survival is probable are caused by damage to the hematopoietic system. In recent years scientific discoveries have slowly advanced the knowledge of the physiological basis underlying the response of the erythropoietic system to ionizing radiation. Nevertheless, a number of problems remain to be studied and certain of these are the subject of this report.

The present experiment was designed to obtain recovery and residual injury data on hematopoietic stem cells of 6 groups of polycythemic rats exposed to one of three relatively low doses (150, 200, and 250 rads) from one of two radiation sources.

The radiation sources were a 250 kVp x-ray generator and the AFRRI-TRIGA Mark F reactor. About 60 percent of the dose produced by the reactor was from gamma rays; the remainder was from neutrons.

In the polycythemic rats, the response of hematological stem cells to erythropoietin as measured by ^{59}Fe uptake in the progeny of these cells, was utilized as a measure of the effects of the irradiation. Peripheral leukocyte counts were obtained to assess competitive stimulation of related blood cells upon the hypothesized multipotential stem cell pool.

It was observed that stem cell injury increases significantly with increasing radiation dose as expressed in the first rapid recovery phase. Although recovery responses are dose dependent, they are independent of the type of radiation and, in general, the RBE of gamma-neutron radiation appears to be 1. A significant decrease in the recovery response is observed in animals subjected to a repeated dose of 200 or 250 rads after a 90-day rest period. Leukopoietic recovery, as noted in the circulatory blood, begins approximately 6 days after exposure.

The data suggest that the postirradiation physiological adjustments in the rat initiated by recovery and subsequent cellular proliferation result in the early abortive cellular rise and its termination.

In this investigation, the residual injury observed after a repeated radiation exposure could be the result of faulty recovery in a number of primitive progenitor cells. However, the possibility of a reduction in numbers due to anatomical changes in the internal environment must be considered.

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